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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Title: METHOD AND MATERIALS FOR INTROGRESSION OF NOVEL GENETIC VARIATION IN MAIZE

Applicant: Mary W. Eubanks

Application No. 10/614,255

Filing Date: 07/03/2003

Examiner: Keith O. Robinson

Group Art Unit: 1638

APPEAL BRIEF

This appeal brief is being filed in follow-up to the Notice of Appeal filed 02 April 2007. A check in the amount of $250 is enclosed in accordance with the 37 CFR 41.20(b)(2) small entity fee requirement for filing a brief in support of an appeal.

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Real Party in Interest (i)

The real party in interest is Mary Wilkes Eubanks, inventor, and pro se applicant.

Related Appeals and Interferences (ii)

None

Status of Claims (iii)

Claims 1-22 cancelled.

Claim 23 (method claim) allowed.

Claims 24-43 (product claims) rejected.

This appeal seeks reversal of rejections to claims 24-43.

Status of Amendments (iv)

In the "Advisory Action Before the Filing of an Appeal Brief," Examiner Keith O. Robinson indicated amended claim 23 (method claim) is allowable. Examiner Robinson indicated claims 24-43 (product claims) remain rejected for the reasons of record. The Examiner Robinson did not stipulate which "reasons of record" are the basis for rejection of the method claims 24-43.

Summary of Claimed Subject Matter (v)

Claim 23, currently amended according to the Examiner's guidance set forth in his facsimile transmission date 23 February 2006, describes a method of identifying a maize plant introgressed with allelic fragments of DNA that correspond to recombinant segments of *Tripsacum*-teosinte chimeric genetic origin and/or segments of *Tripsacum* origin not found in maize (see specification, page 13, lines 29-36 and page 14, lines 1-13). The precise novel restriction fragments claimed are described according to their specific RFLP probe-restriction enzyme combination and molecular weight, and are found in Tables 2 and 3 of the specification (pages 35-48). The Examiner has indicated currently amended claim 23 is allowable (Examiner Robinson's "Advisory Action Before the Filing of an Appeal Brief" and Examiner's "Interview Summary" of telephonic interview 3 April 2007).

The rejected claims 24-43 are method claims dependent upon claim 23. This appeal argues for reversal of the rejections of claims 24-43.

Claims 24-27 relate to hybrid maize seed, hybrid maize plants produced by the seed and/or tissue culture, variants, mutants, modifications, and cellular and molecular components of the hybrid maize plants containing one or more of the novel DNA fragments of claim 23 (see specification page 7, lines 15-20, page 13 lines 17-23, and page 14, lines 13-18). Specific maize lines introgressed with one or more of the fragments in claim 23 are described in the specification (see page 22 lines 21-38). They include: 64SS (W64A X Sun Star), 64TC (W64A X Tripsacorn), 2019 (B73 X Tripsacorn), 4021 (B73 X Tripsacorn), 3024 (B73 X Tripsacorn), 3028 (B73 X Tripsacorn backcrossed to Tripsacorn), 3125 (W64A X Tripsacorn), 4126 (W64A X Tripsacorn), 3029 (B73 X Tripsacorn), 4029 (B73 X Tripsacorn), 10 individuals of TC64 (Tripsacorn X W64A), 7022 (TC64 backcrossed to Tripsacorn), 7024 (Tripsacorn X W64A), 9094 X 7009 (an advanced maize line in a B73/W64A maize background introgressed with Tripsacorn and Sun Star), 97-5 X 97-1 (an advanced maize line in a B73/W64A maize background introgressed with Tripsacorn and Sun Star), and V70 (an advanced maize line in a W64A/A188 maize background introgressed with Tripsacorn and Sun Star). The novel fragments that occur in each of the above-designated maize lines are found in Tables 2 and 3 of the specification (pages 35-48).

Claim 28 is for a plant identified by the method of claim 23 that has improved grain quality (see specification page 1, lines 14-25; page 7, lines 24-30; page 14, lines 19-5, and page 23 lines 35-36 continuing on page 24, lines 1-5).

Claim 29 is for a plant identified by the method of claim 23 that is tolerant of acid soils (see specification page 1, lines 14-24; page 7, lines 24-28, and page 23 lines 35-36 continuing on page 24, lines 1-5).

Claim 30 is for a plant identified by the method of claim 23 that has aflatoxin resistance (see specification page 7, lines 24-30.

Claim 31 is for a plant identified by the method of claim 23 that is resistant to corn borer (see specification page 7, lines 24-26.

Claim 32 is for a plant identified by the method of claim 23 that has aerenchyma in the roots (see specification page 1, lines 14-23; page 7, lines 24-27, and page 27, lines 16-17 and lines 26-31).

Claim 33 is for a plant identified by the method of claim 23 that tolerates saturated soils (see specification page 1, lines 14-24; page 14, lines 19-24, and page 23, lines 35-36 continuing on page 24, lines 1-4).

Claim 34 is for a plant identified by the method of claim 23 that is resistant to aluminum toxicity (see specification page 1, lines 14-24; page 14, lines 19-26, and page 23, lines 35-36 continuing on page 24, lines 1-5).

Claim 35 is for a plant identified by the method of claim 23 that is drought tolerant is (see specification page 1, lines 14-21; page 7, lines 24-26; page 14, lines 19-24; page 23, lines 35-36 continuing on page 24, lines 1-2, and page 27, lines 6-27).

Claim 36 is for a plant identified by the method of claim 23 that has a novel fragment or fragments revealed by SSR probes phi123, bnlg2235, bnlg1714, bnlg1805, dupSSR23 (see specification page 29, lines 1-4 and lines 30-34).

Claim 37 is for a plant identified by the method of claim 23 that has tolerance to low nitrogen (see specification page 1, lines 14-26, and page 14, lines 19-29).

Claim 38 is for a plant identified by the method of claim 23 that exhibits apomixis (see specification page 1, lines 14-22, and page 23, lines 35-36 continuing on page 24, lines 1-3).

Claim 39 is for a plant identified by the method of claim 23 that is cold tolerant (see specification page 1, lines 14-21; page 14, lines 19-24, and page 23, lines 35-36 continuing on page 24, lines 1-2).

Claim 40 is for a plant identified by the method of claim 23 that has improved forage quality (see specification page 1, lines 14-25; page 14, lines 19-25, and page 23, lines 35-36 continuing on page 24, lines 1-5).

Claim 41 is for a plant identified by the method of claim 23 that has more extensive, robust roots (see specification page 1, lines 14-22, and page 23, lines 35-36 continuing on page 24, lines 1-3).

Claim 42 is for a plant identified by the method of claim 23 that exhibits totipotency (see specification page 1, lines 14-22; page 14, lines 19-25, and page 23, lines 35-36 continuing on page 24, lines 1-3).

Claim 43 is for a plant identified by the method of claim 23 that exhibits perennialism (see specification on page 1, lines 14-21; page 7, lines 24-27; page 14, lines 19-25, and page 23, lines 35-36 continuing on page 24, lines 1-2).

Grounds of Rejection to Be Reviewed on Appeal (vi)

In the "Advisory Action Before the Filing of an Appeal Brief," Examiner Keith O. Robinson stated that currently amended claim 23 (method claim) is allowable. Examiner Robinson indicated claims 24-43 (product claims) remain rejected for the reasons of record. The Examiner did not stipulate which "reasons of record" are the basis for rejection of the method claims 24-43. Therefore, this appeal addresses each ground of rejection for the various claims that the Examiner put forth in the Final Office Action, dated 29 November 2006.

In the Examiner's Final Rejection office action, 29 November 2006, claims 24-43 were rejected as follows:

(1) wording informalities – claims 24-43;

(2) written description (35 U.S.C. 112) – claims 23-43;

(3) enablement (35 U.S.C. 112) – claims 23-43;

(4) prior art (35 U.S.C. 102) – claims 24-27.

Argument

Wording Informalities

The Examiner objected to claim 24 because it was dependent on claim 23 as "drawn to a method for transferring a trait of interest and not to a maize plant." (Final Office Action, 29 November 2006, page 3, lines 3-5). Claim 23 was amended according to the Examiner's guidance set forth in his facsimile transmission dated 23 February 2007. Curently amended claim 23 is drawn to "a method of identifying a maize progeny plant" having one or more of the restriction fragments listed in the claim. Claim 23 refers to a method for identifying a plant rather than a method for transferring a trait. Therefore the objections to claims 24-43, which are dependent on claim 23, no longer apply because of the wording change. Claims 24-43 were revised according to the Examiner's instructions to correct the wording informalities from "A plant..." to "The plant..." in the Applicant's Response to Interview Summary, Office Action, and Amendments to Claims, dated 12 August 2006, page 2, lines 13-14.

Written Description (35 U.S.C. 112)

In rejecting claims 24-43 based on the written description, the Examiner said (Final Office Action, 29 November 2006, page 3, lines 19-21): "The claims are broadly drawn to a method for transferring any trait of interest into a maize plant wherein said trait is associated with one or more molecular markers." Currently amended claim 23 is now drawn to "a method of identifying a maize progeny plant" having one or more of the restriction fragments listed in the claim, rather than to a method for transferring a trait. The earlier reasoning for rejecting claims 24-43, which are dependent on claim 23, no longer applies because of the wording change.

In regard to claims 28-31 and 33-43, the Examiner stated (Final Office Action, 29 November 2006, page 4, lines 8-10): "there does not appear to be literal support in the specification for plants with the claimed characteristics." In regard to claims 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, and 43, each trait in the claims is referred to in the specification. Annotation of where support for each trait can be found in the specification per claim follows below and on pages 7-9 of this Appeal Brief under the heading Summary of Claimed Subject Matter. Therefore, the disclosure does provide literal support for the specific

claimed subject matter of each claim, and the Applicant requests reversal of the rejections of claims 24, 25, 26, 27, 28, 29, 30, 31, 32, 35, 36, and 43 based on written description.

Written Description and Enablement (35 U.S.C. 112) - Claim 24

Claim 24 is drawn to a maize seed containing one or more of the restriction fragments of claim 23. Literal support for the claimed subject matter is found in the specification (page 13, lines 17-23 and page 14, lines 13-18). For clarification, this claim is not trait associated.

Written Description and Enablement (35 U.S.C. 112) - Claim 25

Claim 25 is drawn to a maize plant, all derivatives, subsequent generations, variants, mutants, modifications, and cellular and molecular components from a plant containing one or more of the restriction fragments of claim 23. Literal support for the claimed subject matter is found in the specification (page 13, lines 17-28 and page 14, lines 13-18). For clarification, this claim is not trait associated.

Written Description and Enablement (35 U.S.C. 112) - Claim 26

Claim 26 is drawn to pollen from a plant containing one or more of the restriction fragments of claim 23. Literal support for the claimed subject matter is found in the specification (page 13, lines 17-28 and page 14, lines 13-18). For clarification, this claim is not trait associated.

Written Description and Enablement (35 U.S.C. 112) - Claim 27

Claim 27 is drawn to tissue cultures, all derivatives, variants, mutants, modifications, and cellular and molecular components from a plant containing one or more of the restriction fragments of claim 23. Literal support for the claimed subject matter is found in the specification (page 13, lines 17-28 and page 14, lines 13-18). For clarification, this claim is not trait associated.

Written Description and Enablement (35 U.S.C. 112) - Claim 28

Claim 28 is drawn to a plant containing one or more of the restriction fragments of claim 23 that has improved grain quality. Literal support for the claimed subject matter is found in the

specification on page 1, lines 14-25; page 7, lines 24-30; page 14, lines 19-5, and on page 23 lines 35-36 continuing on page 24, lines 1-5.

Written Description and Enablement (35 U.S.C. 112) - Claim 29

Claim 29 is drawn to a plant containing one or more of the restriction fragments of claim 23 that tolerates acid soil. Thus it is drawn to a particular trait and is not broadly drawn to a method for transferring any trait of interest into a maize plant. Literal support for the claimed subject matter is found in the specification on page 1, lines 14-24; page 7, lines 24-28, and page 23 lines 35-36 continuing on page 24, lines 1-5.

Written Description and Enablement (35 U.S.C. 112) – Claim 30

Claim 30 is drawn to a plant containing one or more of the restriction fragments of claim 23 that is resistant to aflatoxin. Thus it is drawn to a particular trait and is not broadly drawn to a method for transferring any trait of interest into a maize plant. Literal support for this claim is found in the specification on page 7, lines 24-30.

Written Description and Enablement (35 U.S.C. 112) – Claim 31

Claim 31 is drawn to a plant containing one or more of the restriction fragments of claim 23 that is resistant to corn borer. Thus it is drawn to a particular trait and is not broadly drawn to a method for transferring any trait of interest into a maize plant. Literal support for this claim is found in the specification on page 7, lines 24-26.

Written Description and Enablement (35 U.S.C. 112) – Claim 32

Claim 32 is drawn to a plant containing one or more of the restriction fragments of claim 23 that has aerenchyma in its roots. Thus it is drawn to a particular trait and is not broadly drawn to a method for transferring any trait of interest into a maize plant. Literal support for this claim that shows the applicant was in possession of the specific subject matter claimed is found in the specification on page 1, lines 14-23; page 7, lines 24-27, and page 27, lines 16-17 and lines 26-31.

Written Description and Enablement (35 U.S.C. 112) – Claim 33

Claim 33 is drawn to a plant containing one or more of the restriction fragments of claim 23 that tolerates saturated soils. Thus it is drawn to a particular trait and is not broadly drawn to a method for transferring any trait of interest into a maize plant. Literal support for this claim is found in the specification on page 1, lines 14-24; page 14, lines 19-24, and page 23, lines 35-36 continuing on page 24, lines 1-4.

Written Description and Enablement (35 U.S.C. 112) – Claim 34

Claim 34 is drawn to a plant containing one or more of the restriction fragments of claim 23 that is resistant to aluminum toxicity. Thus it is drawn to a particular trait and is not broadly drawn to a method for transferring any trait of interest into a maize plant. Literal support for this claim is found in the specification on page 1, lines 14-24; page 14, lines 19-26, and page 23, lines 35-36 continuing on page 24, lines 1-5.

Written Description and Enablement (35 U.S.C. 112) – Claim 35

Claim 35 is drawn to a plant containing one or more of the restriction fragments of claim 23 that is drought tolerant. Thus it is drawn to a particular trait and is not broadly drawn to a method for transferring any trait of interest into a maize plant. Literal support for this claim that shows the applicant was in possession of the specific subject matter claimed is found in the specification on page 1, lines 14-21; page 7, lines 24-26; page 14, lines 19-24; page 23, lines 35-36 continuing on page 24, lines 1-2, and page 27, lines 6-27.

Written Description and Enablement (35 U.S.C. 112) – Claim 36

Claim 36 is drawn to a plant containing one or more of the restriction fragments of claim 23 further comprising novel bands identified by SSR probes phi123, bnlg2235, bnlg1714, bnlg1805, and dupSSR23. Thus it is drawn to particular SSR probes whose genetic loci correspond to the same loci as specific RFLP markers designated in claim 23, and is not broadly drawn to a method for transferring any trait of interest into a maize plant. Literal support for this claim that shows the applicant was in possession of the claimed subject matter claimed is found in the specification on page 29, lines 1-4 and lines 30-34.

Written Description and Enablement (35 U.S.C. 112) – Claim 37

Claim 37 is drawn to a plant containing one or more of the restriction fragments of claim 23 that has tolerance to low nitrogen. Thus it is drawn to a particular trait and is not broadly drawn to a method for transferring any trait of interest into a maize plant. Literal support for this claim is found in the specification on page 1, lines 14-26, and page 14, lines 19-29.

Written Description and Enablement (35 U.S.C. 112) – Claim 38

Claim 38 is drawn to a plant containing one or more of the restriction fragments of claim 23 that exhibits apomixis. Thus it is drawn to a particular trait and is not broadly drawn to a method for transferring any trait of interest into a maize plant. Literal support for this claim is found in the specification on page 1, lines 14-22, and page 23, lines 35-36 continuing on page 24, lines 1-3.

Written Description and Enablement (35 U.S.C. 112) – Claim 39

Claim 39 is drawn to a plant containing one or more of the restriction fragments of claim 23 that is cold tolerant. Thus it is drawn to a particular trait and is not broadly drawn to a method for transferring any trait of interest into a maize plant. Literal support for this claim is found in the specification on page 1, lines 14-21; page 14, lines 19-24, and page 23, lines 35-36 continuing on page 24, lines 1-2.

Written Description and Enablement (35 U.S.C. 112) – Claim 40

Claim 40 is drawn to a plant containing one or more of the restriction fragments of claim 23 that has improved forage quality. Thus it is drawn to a particular trait and is not broadly drawn to a method for transferring any trait of interest into a maize plant. Literal support for this claim is found in the specification on page 1, lines 14-25; page 14, lines 19-25, and page 23, lines 35-36 continuing on page 24, lines 1-5.

Written Description and Enablement (35 U.S.C. 112) – Claim 41

Claim 41 is drawn to a plant containing one or more of the restriction fragments of claim 23 that has more extensive, robust roots. Thus it is drawn to a particular trait and is not broadly drawn to a method for transferring any trait of interest into a maize plant. Literal support for this

claim is found in the specification on page 1, lines 14-22, and page 23, lines 35-36 continuing on page 24, lines 1-3.

Written Description and Enablement (35 U.S.C. 112) – Claim 42

Claim 42 is drawn to a plant containing one or more of the restriction fragments of claim 23 that exhibits totipotency. Thus it is drawn to a particular trait and is not broadly drawn to a method for transferring any trait of interest into a maize plant. Literal support for this claim is found in the specification on page 1, lines 14-22; page 14, lines 19-25, and page 23, lines 35-36 continuing on page 24, lines 1-3.

Written Description and Enablement (35 U.S.C. 112) – Claim 43

Claim 43 is drawn to a plant containing one or more of the restriction fragments of claim 23 exhibits perennialism. Thus it is drawn to a particular trait and is not broadly drawn to a method for transferring any trait of interest into a maize plant. Literal support for this claim that shows the applicant was in possession of the specific subject matter claimed is provided in the specification on page 1, lines 14-21; page 7, lines 24-27; page 14, lines 19-25, and page 23, lines 35-36 continuing on page 24, lines 1-2. Perennial maize X *Tripsacum*-teosinte hybrids that have been growing in the applicant's greenhouse since the 1990's are TC64 and 7022 (described in the specification on page 22, lines 23-29). DNA profiles of TC64 and 7022 are included in Tables 2 and 3 of the specification (pages 35-48). This evidence verifies such plants existed and were possessed by the applicant at the time of the application.

Enablement (35 U.S.C. 112)

In the enablement rejection (35 U.S.C. 112) for claims 24-43, the Examiner said (Final Office Action, 29 November 2006, page 8, lines 15-16): "The claims are broadly drawn to a method for transferring any trait of interest into a maize plant wherein said trait is associated with one or more molecular markers." Currently amended claim 23 is now drawn to "a method of identifying a maize progeny plant" having one or more of the restriction fragments listed in claim 23 and is not drawn to a method for transferring a trait. Therefore, the earlier objection to claims 24-43, which are dependent on claim 23, no longer stands because of the wording change.

In regard to claims 28-31 and 33-43, the Examiner stated (Final Office Action, 29 November 2006, page 9, lines 5-9): "it would require undue trial and error experimentation for one skilled in the art to make and use the claimed invention because one skilled in the art would not know which markers are associated with any particular trait."

According to the M.P.E.P 2164.01 Test of Enablement [R-5] (page 2100-187, column 1, lines 16-21), "The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation." Under the heading Undue Experimentation (M.P.E.P. 2164.01, page 2100-187, column 1) the statue reads: "The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation."

The disclosure describes a simple process of rapid detection of alleles associated with a trait of interest by comparing the molecular weights of the probe/enzyme fragments in progeny expressing a particular agronomic trait to progeny that do not express that trait. This invention does not require undue experimentation. To the contrary, it bypasses undue experimentation needed to develop maize linkage maps using molecular markers to identify which genetic loci correlate with specific phenotypic traits. This invention avoids the extremely large plant populations, multiple generations, DNA fingerprinting of large numbers of individuals, and complex statistical analyses of hundreds of plants that are required for finding a molecular marker-trait association in maize linkage mapping.

The disclosure distinctly enables one skilled in the art to make a cross between *Tripsacum* sp. and teosinte (page 12, lines 22-25) and they will recover recombinant plants that will contain one or more novel alleles presented in claim 23 and described in Tables 2 and 3 of the specification (pages 35-48). It further enables one skilled in the art to cross those plants with maize as described on page 13 of the specification (lines 17-23), and those progeny will contain one or more of the novel alleles in claim 23 and Tables 2 and 3 of the specification. It further enables one skilled in the art to make backcrosses to maize (page 14, lines 8-13), and those progeny will contain one or more of the novel alleles in claim 23 and Tables 2 and 3 of the specification. The DNA fingerprinting process to obtain a restriction fragment profile of a plant is described in the specification (see page 12, lines 9-21 continued on page 13, lines 1-9). A simple process of comparing the banding patterns of the novel alleles (i.e. molecular weights per probe-enzyme combination) in a progeny expressing a particular trait to a progeny that does not

have the trait permits rapid detection of alleles associated with the trait of interest. It therefore follows that one can use the molecular marker fragments identified in a plant by the method of claim 23, which are also found to be associated with a trait, to screen young seedlings and identify the plants that possess a trait of interest without having to grow out large populations to maturity to determine phenotypic expression of the presence or absence of the trait of interest.

Further citing the M.P.E.P. 2164.02 Working Example Enablement Requirement [R-2] page 2100-189: "Compliance with the enablement requirement of 35 U.S.C. 112, first paragraph, does not turn on whether an example is disclosed. An example may be 'working' or 'prophetic.' A working example is based on work actually performed. A prophetic example describes an embodiment of the invention based on predicted results rather than work actually conducted or results actually achieved. An applicant need not have actually reduced the invention to practice prior to filing." In *Gould v. Quigg*, 822 F2d 1074, 1078, 3 USPQ 2d 1302, 1304 (Fed. Cir. 1987). "The specification need not contain an example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without undue experimentation." *In re Borkowski*, 422 F2d 904, 908, 164 USPQ 642, 645 (CCPA 1970). This specification provides two working examples that teach how to determine the association of novel alleles from claim 23 with a trait in maize such that one skilled in the art could easily apply the technique to identify particular fragment-trait associations without undue experimentation.

Further citing the M.P.E.P. 2164 Enablement Requirement [R-2] page 2100-186: "...to comply with 35 U.S.C. 112, first paragraph, it is not necessary to "enable one of ordinary skill in the art to make and use a perfected, commercially viable embodiment absent a claim limitation to that effect." The specification provides two clear working examples of precisely how to determine which fragments are associated with two different traits; corn rootworm resistance, which is a polygenic trait (specification page 24, lines 23-36 continued on page 25, lines 1-15), and root aerenchyma, which is a single gene trait (page 27, lines 16-36 continuing on page 28, lines 1-3 and lines 28-36 continuing on page 29, lines 1-4). The Examiner acknowledged (Final Office Action 29 November 2006, page 11, lines): "The specification provides guidance and working examples regarding the claimed invention using RFLPs associated with corn rootworm resistance and aerenchyma." Following these examples, anyone skilled in the art could rapidly determine whether a trait of interest is associated with one or more of the novel restriction fragments listed in claim 23. The examples in the disclosure contain sufficient information to

enable one skilled in the pertinent art to make and use the claimed invention and to detect fragments that are associated with a particular trait. The traits designated in claims 28-43 are traits that are known in *Tripsacum* and have been observed among segregant maize progeny plants in the applicant's breeding program. The above information is deemed to satisfy the 35 U.S.C. 112 enablement requirement, and the applicant requests reversal of the enablement (35 U.S.C. 112) rejections of claims 24-43.

Prior art (35 U.S.C. 102)

The Examiner rejected claims 24-27 under 35 U.S.C. 102 as being anticipated by Eubanks (U.S. Patent 5,330,547, July 19, 1994). The Examiner argued (page 12, lines 8-11 of the Final Office Action, 11/29/06): 'Though Eubanks does not further screen the plant's DNA as stated in step (f), the plant would inherently possess the claimed restriction fragments because the plant was produced by the same method as the claimed invention."

Citing the Manual of Patent Examining Procedures (M.P.E.P), item IV, page 2100-47: "The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic." *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) "(reversed rejection because inherency was based on what would result due to optimization of conditions, not what was necessarily present in the prior art);" *In re Oelrich*, 666 F.2d 578, 581-82, 212 USPQ 323, 326 (CCPA 1981). "To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.'"

Tables 2 and 3 of the disclosure for this application no. 10/614,255 (pages 35-46) present the results of DNA screening of a (*Tripsacum* X teosinte) plant, as claimed in Eubanks (U.S. Patent 5,330,547, July 19, 1994), referred to as Tripsacorn. It also presents DNA profiles of three other (teosinte X *Tripsacum*) hybrids, designated Sun Dance, 20A, and Sun Star, as well as fourteen examples of specific maize X *Tripsacum*-teosinte recombinant lines. To satisfy the inherency rule, the Tripsacorn recombinant in U.S. Patent 5,330,547 should possess all the fragments in Tables 2 and 3 of the disclosure (pages 35-48). Comparison of the fragments found

in Tripsacorn with the fragments found in the other teosinte-*Tripsacum* hybrids and maize X *Tripsacum*-teosinte hybrids in Tables 2 and 3 of the specification reveals that Tripsacorn and (maize X Tripsacorn) plants claimed in U.S. Patent 5,330,547 do not necessarily possess the restriction fragments of claim 23.

For example, in the first probe/enzyme entry for BNL5.62-ERI in Table 2 of the specification (page 35, line 3), the (teosinte X *Tripsacum)* plant Sun Dance has a 10.3 kb fragment that does not occur in Tripsacorn, 20A, or Sun Star. The same 10.3 kb fragment is found in three out of the fourteen different (maize X *Tripsacum*-teosinte) hybrids, 2019, 3024, and 3028. This illustrates that a fragment does not necessarily occur in any particular plant derived by crossing *Tripsacum* and teosinte or by crossing a *Tripsacum*-teosinte hybrid with maize, or by backcrossing a maize plant introgressed with alleles from a *Tripsacum*-teosinte-maize. Another example underscores this point. Under the probe/enzyme UMC157-B in Table 2 (page 35, line 8), a 5.0 kb fragment is inherited in 20A and Sun Star, but is not present in Tripsacorn or Sun Dance. The same 5.0 kb fragment occurs in the (maize X *Tripsacum*-teosinte) lines 3022 and 7022, but is not found in any of the other *Tripsacum*-teosinte introgressed maize lines. By comparing all the probe/enzyme fragments for all the plant lines listed in Tables 2 and 3 of the specification (pages 35-48), it is evident that the fragments of claim 23 may occur but are not necessarily present in the plants of U.S. Patent 5,330,547. Therefore, the prior art products do not necessarily or inherently possess the characteristics of the products in claims 24-43. These examples from Table 2 of the disclosure (page 35) support the applicant's argument for reversal of the Examiner's prima facie case for inherency.

Citing the M.P.E.P, item IV, page 2100-48, column one, lines 17-21: "In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." The above examples demonstrate that the plant described in Patent No. 5,330,547 and its progeny will not necessarily or inherently possess a fragment listed in claim 23. Nor will those plants possess any trait of interest in the absence of the molecular marker fragments found to be associated with the trait of interest. The method of claim 23 provides a direct way to identify which maize progeny plants possess particular allelic fragments, and it further provides a way to detect which fragments are associated with the maize phenotype of an agronomic trait of interest. The Applicant points out that the traits claimed in

U.S. Patent 5,330,547 are resistance to corn rootworm (claim 8) and lodging (claim 9). Neither of those traits are claimed in this application. The Applicant therefore requests reversal of the 35 U.S.C. 102 rejection of claims 24-27 because the plants in Patent No. 5,330,547 do not necessarily or inherently possess the novel fragments listed in claim 23.

Obviousness (35 U.S.C. 103)

The Examiner is on record that Claim 23 is allowable (ref. Examiner Robinson's Advisory Action Before the Filing of an Appeal Brief and Examiner's Interview Summary of telephone interview 3 April 2007). The applicant's argument in the Response to Final Office Action and Amendments to Claims document filed 26 February 2007 overcame the 35 U.S.C. 103 obviousness rejection. The applicant submits the same reasoning that overcame the Examiner's rejection of method claim 23 likewise overcomes any outstanding 35 U.S.C. 103 obviousness rejections of claims 24-43 (Examiner's first Final Office Action dated 13 March 2006, page 3, lines 12-26 continuing on page 4, lines 1-4).

To summarize the applicant's point that overcomes the 35 U.S.C. 103 obviousness rejection: in a homozygous maize inbred line an RFLP marker detects one or two alleles (i.e. fragments) at a genetic locus in an easily detected pattern. Each maize parent contributes the same parental allele(s) per locus to the hybrid progeny produced by crossing two inbred lines. This invention is completely different and would not have been expected based on prior art. Rather than inheriting one or two alleles (i.e. fragments) from each parent as in maize, the progeny of crosses between *Tripsacum* and teosinte exhibit novel fragments that are not found in either parent. This is unprecedented in the literature, defies the conventional paradigm for allelic inheritance in genetics, and clearly establishes that the prior art would neither anticipate nor render obvious the claimed invention.

No molecular data are presented or claimed in Eubanks (U.S. Patent 5,330,547, 1993). One of ordinary skill in the art would have expected the DNA fingerprints of *Tripsacum*-teosinte recombinant plants in U.S. Patent 5,330,547 to show that hybrid progeny inherited two to four parental alleles at each genetic locus. The prior art could not have anticipated the formation of a large number of novel intergeneric fragments (i.e. novel alleles) and that those novel allelic fragments could be stably inherited in maize. Therefore, obviousness could not have been predicated on what was known at the time of U.S. Patent 5,330,547.

In the the Examiner's Office Action, dated 6 June 2006, Conclusion section, page 12, under item 14, the Examiner stated that "Claims 13, 16, 17, 18, 19, 20, and 22 are deemed free of prior art given the failure of the prior art to teach or suggest a maize plant that contains one or more restriction fragments, produced by crossing a *Tripsacum*/teosinte hybrid with a maize plant, and wherein the hybrid from said cross is distinguished by the presence of..." the traits designated in each of those claims, respectively. The claims previously deemed free of prior art correspond to currently amended claims as follows: claim 13 corresponds to claim 32; claim 16 corresponds to claim 28; claim 17 corresponds to claim 29; claim 18 corresponds to claim 30; claim 19 corresponds to claim 31, and claim 22 corresponds to claim 37.

The arguments and evidence presented herein provide support for the Applicant's request the rejections of claims 24-43 be reversed and those product claims be allowed.

Claims Appendix

Claims 1-22 (Canceled).

Claim 23 (Currently amended). A method of identifying a maize progeny plant having a restriction fragment introgressed from a *Tripsacum*/teosinte hybrid, said method comprising the following steps:

(a) isolating the total genomic DNA from the plant;

(b) digesting said genomic DNA with one to five of the restriction enzymes selected from the group consisting of *Eco*RI, *Eco*RV, *Hind*III, *Bam*HI and *Msp*I;

(c) probing said digested genomic DNA with one or more probes, to identify one or more restriction fragments, selected from the group consisting of

BNL5.62, *Eco*RI, 10.3 kb; npi97, *Hind*III, 3.9 kb; UMC157, *Eco*RI, 6.5 kb and 3.3 kb; UMC157, *Hind*III, 5.5 kb; UMC157, *Bam*HI, 14.0 kb, 8.5 kb and 4.5 kb; UMC11, *Bam*HI, 7.0 kb; CSU3, *Bam*HI, 10.0 kb and 7.6 kb; UMC67, *Eco*RI, 19.2 kb; UMC67, *Bam*HI 13.4 kb, 11.0 kb and 1.6 kb; CSU92, *Bam*HI, 13.3 kb and 7.5 kb; asg62, *Bam*HI, 12.7 kb, 9.7 kb and 6.6 kb; UMC58, *Hind*III, 3.3 kb; CSU164, *Eco*RI, 9.0 kb and 7.0 kb; UMC128, *Hind*III, 6.0 kb; UMC107, *Eco*RI, 7.5.0 kb, 6.3 kb and 6.1 kb; UMC140, *Eco*RI, 4.9 kb; UMC140, *Hind*III, 6.5 kb; adh1, *Hind*III, 9.4 kb; adh1, *Bam*HI, 9.4 kb; UMC161, *Hind*III, 3.3 kb; BNL8.29, *Hind*III, 9.3 kb and 8.3 kb; UMC53, *Eco*RI, 9.4 kb; UMC53, *Eco*RV, *8.4 kb*, 3.8 kb and 3.0 kb; UMC6, *Eco*RI, 3.8 kb; UMC6, *Hind*III 9.4 kb; UMC6, *Bam*HI, 13.2 kb, 12.7 kb, and 7.0 kb; UMC61, *Hind*III, 3.4 and 2.8 kb *agrr167*, *Bam*HI, 5.7 kb, 4.5 kb and 4.0 kb; UMC34, *Eco*RI, 7.5 kb and 5.4 kb; UMC34, *Hind*III, 8.8 kb, 6.5 kb and 5.8 kb; UMC34, *Bam*HI, 9.4 kb; UMC135, *Hind*III, 11.6 kb and 10.8 kb; UMC131, *Eco*RI, 10.6 kb, 5.8 kb and 4.3 kb; UMC55, *Eco*RI, 3.9 kb; UMC55, *Hind*III, 4.3

kb; UMC5, *Eco*RI, 5.4 kb; UMC5, *Hind*III, 6.5 kb; UMC49, *Bam*HI, 8.2 kb; UMC36, *Bam*HI, 4.2 kb; UMC32, *Eco*RI, 5.3 kb; UMC32, *Hind*III 6.7 kb, 6.0 kb, and 2.8 kb; asg24, *Hind*III, 7.2 kb and 6.4 kb; UMC121, *Eco*RI, 3.7 kb and 3.2 kb; BNL8.35, *Hind*III, 9.9 kb and 8.7 kb; *UMC50*, *Bam*HI, *7.8* kb, 6.8 kb, *5.8* kb and 3.8 kb; *UMC42*, *Hind*III, 10.4 kb, 9.2 kb, 8.9 kb, 7.9 kb, *7.6* kb, and 3.7 kb; npi247, *Eco*RI, 8.0 kb; npi247, *Hind*III 3.0 kb; UMC10, *Hind*III, 3.0 kb; UMC10, *Eco*RI, 6.5 kb and 5.5 kb; UMC102, *Eco*RI, 2.7 kb; BNL6.06, *Eco*RI, 6.8 kb; CSU240, *Eco*RI, 10.6 kb, 4.5 kb and 3.3 kb; BNL5.37, *Hind*III, 10.3 kb, 5.8 kb and 3.5 kb; npi296, *Eco*RI, 7.9 kb; UMC3, *Eco*RI 2.5 kb and 2.0 kb; npi212, *Hind*III, 4.3 kb; npi212, *Bam*HI, 5.4 kb; UMC39, *Eco*RI, 12.2 kb, 9.2 kb, 7.8 kb and 7.1 kb; *phi10080*, *Bam*HI, *9.7* kb; UMC63, *Hind*III, 9.5 kb and 4.3 kb; CSU303, *Eco*RI, 10.0 kb; UMC96, *Hind*III, 11.8 kb, 6.4 kb and 5.5 kb; UMC96, *Bam*HI, 7.5 kb; UMC2, *Eco*RI, 11.8 kb, 10.4 kb, 8.0 kb and 3.9 kb; *CSU25*, *Hind*III, *5.2* kb, 4.5 and *4.2* kb; agrr115, *Eco*RI. 8.0 kb and 5.4 kb; agrr115, *Bam*HI, 5.4 kb and 3.5 kb; phi20725, *Eco*RI, 10.3 kb, 9.7 kb and 7.2 kb; phi20725, *Hind*III, 1.5 kb; UMC31, *Eco*RI, 5.8 kb and 2.0 kb; *UMC31*, *Bam*HI 6.5 kb; UMC55, *Eco*RI, 3.9 kb; UMC55, *Hind*III, 4.3 kb; CSU235, *Hind*III, 6.8 kb and 3.0 kb; CSU585, *Hind*III, 8.3 kb and 6.1 kb; BNL5.46, *Hind*III, 13.7 kb, 10.5 kb, 9.7 kb and 5.1 kb; agrr321, *Bam*HI, 5.5 kb; *agrr89*, *Hind*III, *7.1* kb; npi386, *Hind*III, 12.6 kb, 9.3 kb and 8.2 kb; *UMC42*, *Hind*III, 19.2 kb, 10.3 kb 8.9 kb, *7.6* kb, 3.7 kb and 3.0 kb; *tda62*, *Bam*HI, 5.5 kb, 5.2 kb, 4.8 kb and *4.2* kb; BNL5.71, *Eco*RV, 11.3 kb, 6.8 kb, and 5.7 kb; UMC156, *Hind*III, 3.0 kb; UMC66, *Eco*RI, 10.5 kb; UMC66, *Bam*HI, 3.7 kb and 2.4 kb; UMC19, *Bam*HI, 12.3 kb; UMC104, *Hind*III, 12.4 kb, 11.6 kb and 7.5 kb; UMC104, *Bam*HI, 9.4 kb; UMC133, *Hind*III, 10.6 kb, 9.9 kb, 9.2 kb and 7.7 kb; UMC52, *Bam*HI, 8.7 kb, 6.9 kb, 3.8 kb, 3.0 kb and 2.0 kb; BNL15.07, *Hind*III, 2.9 kb and 2.7 kb; npi409, *Eco*RI, 9.4 kb; npi409, *Hind*III, 10.4 kb, 9.0 kb and 3.9 kb; UMC147, *Hind*III, 16.3 kb, 3.8 kb and 2.4 kb; *asg73*, *Eco*RI,

3.8 kb; UMC90, *Hind*III, 7.7 kb, 6.5 kb, 2.8 kb and 1.6 kb; UMC90, *Bam*HI, 9.0 kb; *UMC72*, *8.5* kb; UMC27, *Hind*III, 8.3 kb and 4.5 kb; UMC27, *Bam*HI, 6.5 kb; UMC43, *Bam*HI, 9.7 kb, 7.3 kb and 5.7 kb; tda37, *Bam*HI, 9.0 kb, 8.0 kb and 6.4 kb; UMC43, *Bam*HI, 9.7 kb, 7.3 kb and 5.7 kb; UMC40, *Bam*HI, 7.2 kb, 4.7 kb and 4.3 kb; BNL7.71, *Hind*III, 10.6 kb; BNL5.71, *Bam*HI, 11.3 kb, 6.8 kb and 5.7 kb; tda62, *Bam*HI, 6.5 kb and 5.5 kb; UMC68, *Hind*III, 6.0 kb; UMC104, *Hind*III, 12.4 kb, 11.6 kb and 7.5 kb; UMC104, *Bam*HI, 9.4 kb; phi10017, *Bam*HI, 15.1 kb and 9.5 kb; tda50, *Bam*HI, 8.5 kb; npi373, *Hind*III, 6.5 kb, 5.6 kb, 5.1 kb and 3.0 kb; tda204, *Bam*HI, 4.0 kb; *npi393*, *Eco*RI, 12.1 kb, 8.5 kb, *7.0* kb and 5.6 kb; UMC65, *Hind*III, 2.9 kb; UMC46, *Eco*RI, 6.5 kb and 5.6 kb; asg7, *Hind*III, 6.3 kb; UMC28, HindIII, 15.8 kb and 11.9 kb; UMC28, *Bam*HI, 9.9 kb, 7.6 kb and 6.6 kb; UMC134, *Hind*III, 7.5 kb and 4.7 kb; *asg8*, *Hind*III, 10.8 kb, *8.7* kb and 8.4 kb; *phi20581*, *Hind*III, *4.2* kb; O2, *Eco*RI, 9.4 kb; asg34, *Hind*III, 4.5 kb; BNL15.40, *Hind*III, 5.8 kb; UMC116, *Eco*RI, 9.5 kb; *UMC110*, *Bam*HI, 10.6 kb, 4.9 kb and *3.9* kb; BNL8.32, *Hind*III, 8.9 kb, 7.4 kb and 7.1 kb; BNL14.07, *Eco*RI, 6.4 kb; *UMC80*, *Hind*III, *10.7* kb, *8.2* kb and 2.4 kb; BNL16.06, *Eco*RI, 6.8 kb and 1.9 kb; BNL16.06, *Hind*III, 5.7 kb, 3.0 kb and 1.6 kb; phi20020, *Hind*III, 7.8 kb, 6.6 kb and 5.1 kb; npi114, *Hind*III, 10.0 kb, 8.8 kb and 6.3 kb; BNL9.11, *Hind*III, 3.4 kb; UMC103, *Hind*III, 6.9 kb; UMC124, *Hind*III, 8.0 and 7.0; UMC124, BamHI, 6.6 kb, 2.6 kb and 1.6 kb; UMC120, *Hind*III, 3.2 kb, 2.3 kb and 1.4 kb; UMC89, *Eco*RI, 7.3 kb; UMC89, *Hind*III, 7.3 kb; UMC89, *Bam*HI, 9.5 kb, 6.0 kb, 5.2 kb and 4.5 kb; UMC89, *Msp*I, 6.7 kb and 5.8 kb; BNL12.30, *Eco*RI, 3.5 kb; *UMC48*, *Hind*III, *6.2* kb, 5.3 kb, 4.7 kb, 4.2 kb and 3.5 kb; UMC53, *Eco*RI, 3.8 kb and 3.0 kb; *UMC53*, *Eco*RV, 8.4 kb; npi268, *Bam*HI, 6.4 kb; *UMC7*, *Bam*HI, 4.2 kb; UMC3, *Eco*RI, 3.5 kb and 2.0 kb; phi10005, *Eco*RI, 15.0 kb and 1.6 kb; UMC113, *Eco*RI, 5.9 kb and 5.4 kb; UMC113, *Bam*HI, 12.8 kb, 11.8 kb and 10.5 kb; UMC192, *Hind*III, 11.4 kb and 6.4 kb; wx (waxy), *Hind*III, 21.0 kb; UMC105,

*Eco*RI, 3.9 kb; CSU147, *Hind*III 5.9 kb; BNL5.10, *Hind*III, 6.1 kb and 4.4 kb; UMC114, *Bam*HI, 12.6 kb, 11.5 kb, 10.0 kb, 8.8 kb, 7.5 kb and 6.5 kb; UMC95, *Eco*RI, 5.6 kb; UMC95, *Hind*III, 7.7 kb, 7.3 kb, 4.8 kb, 4.5 kb 4.1 kb and 1.7 kb; UMC95, *Bam*HI, 15.0 kb and 9.0 kb; asg44, *Eco*RI, 5.3 kb; CSU61, *Eco*RI, 8.1 kb and 4.8 kb; BNL7.57, *Bam*HI, 11.6 kb and 5.9 kb; CSU54, *Eco*RI, 14.7 kb and 12.6 kb; phi20075, *Eco*RI, 7.1 kb; npi285, *Eco*RI, 12.4 kb, 9.4 kb and 6.0 kb; KSU5, *Eco*RI, 9.8 kb, 7.6 kb, 6.1 kb, 3.8 kb and 3.5 kb; UMC130, *Eco*RI, 13.5 kb and 7.0 kb; UMC130, *Hind*III, 4.8 kb and 3.2 kb; UMC130, *Bam*HI, 3.2 kb; UMC64, *Hind*III, 3.3 kb; UMC152, *Hind*III, 12.4 kb, 7.1 kb and 5.6 kb; phi06005, *Eco*RI, 12.8 kb; *UMC163*, *Hind*III, 7.0 kb, 4.8 kb; 3.0 kb; *2.6* kb and 2.3 kb; UMC44, *Hind*III, 9.8 kb, 8.7 kb, 7.2 kb, 5.5 kb and 4.0 kb; BNL10.13, *Hind*III, 10.8 kb; npi306, *Hind*III, 7.0 kb; pmt1, *Hind*III, 2.3 kb; pmt2, *Hind*III, 2.8 kb and 2.1 kb; *pmt5*, *Hind*III, 12.3 kb, 8.1 kb, *3.6* kb, 3.2 kb and 2.5 kb; tda48, *Hind*III, 8.2 kb; tda53, *Hind*III, 3.8 kb and 2.2 kb; tda168, *Eco*RI, 3.6 kb; tda16, *Hind*III, 4.3 kb; and tda17, *Hind*III, 7.0 kb; tda250, *Bam*HI, 4.0 kb, recited as marker-enzyme fragment size;

(d) determining the presence of one or more of the restriction fragments.

Claim 24 (Currently amended). A maize seed identified by the method according to claim 23.

Claim 25 (Currently amended). A maize plant, all derivatives, subsequent generations, variants, mutants, modifications, and cellular and molecular components identified according to the method of claim 23 or produced from the seed according to claim 24.

Claim 26 (Currently amended). The pollen from a plant identified according to the method of claim 23 or according to claim 25.

Claim 27 (Currently amended). The tissue cultures, all derivatives, variants, mutants, modifications, and cellular and molecular components from the plant identified according to the method of claim 23 or according to claim 25.

Claim 28 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 whereby said plant has improved grain quality.

Claim 29 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 whereby said plant is tolerant of acid soils.

Claim 30 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 whereby said plant is resistant to aflatoxin.

Claim 31 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 whereby said plant is resistant to corn borer.

Claim 32 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 whereby the roots of said plant contain aerenchyma.

Claim 33 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 whereby said plant tolerates saturated soils.

Claim 34 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 whereby said plant is resistant to aluminum toxicity.

Claim 35 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 whereby said plant is drought tolerant.

Claim 36 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 further comprising a novel band identified by SSR probe phi123, SSR probe bnlg2235, SSR probe bnlg1714, SSR probe bnlg1805, or SSR probe dupSSR23, thereof.

Claim 37 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 whereby said plant has tolerance to low nitrogen.

Claim 38 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 whereby said plant exhibits apomixis.

Claim 39 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 whereby said plant is cold tolerant.

Claim 40 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 whereby said plant has improved forage quality.

Claim 41 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 whereby said plant has more extensive, robust roots.

Claim 42 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 whereby said plant exhibits totipotency.

Claim 43 (Currently amended). The plant identified according to the method of claim 23 or according to claim 25 whereby said plant exhibits perennialism.

Evidence Appendix

(1) Eubanks, M. W., 1994, Methods and Materials for Conferring *Tripsacum* Genes in Maize. U.S. Patent No. 5,330,547, issued 19 July 1994. (Entered into the record in the Examiner's Interview Summary dated 6 June 2006 and discussed in the Examiner's Office Action dated 16 June 2006, page 12, line 5).

Related Proceedings Appendix

None

US005330547A

United States Patent [19]

Eubanks

[11] Patent Number: 5,330,547

[45] Date of Patent: * Jul. 19, 1994

[54] METHODS AND MATERIALS FOR CONFERRING TRIPSACUM GENES IN MAIZE

[76] Inventor: Mary W. Eubanks, 4110 Hulon Dr., Durham, N.C. 27705

[*] Notice: The portion of the term of this patent subsequent to Sep. 15, 2009 has been disclaimed.

[21] Appl. No.: 944,389

[22] Filed: Sep. 14, 1992

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 613,269, Nov. 13, 1990, Pat. No. P.P. 7,977.

[51] Int. Cl.[5] A01H 1/02; A01H 5/00

[52] U.S. Cl. 47/58; 800/200; 800/DIG. 56; 435/240.4

[58] Field of Search ..... Plt. 100; 47/58.03; 435/240.4, 240.5; 800/200, DIG. 56

[56] References Cited

U.S. PATENT DOCUMENTS

4,659,668	4/1987	Sondahl et al.	435/240.5
4,677,246	6/1987	Armond et al.	800/200

OTHER PUBLICATIONS

de Wet (1979) Proc. Conf. Broadening Genet. Base Crops, Wageningin 1978, pp. 203–210.

Primary Examiner—Che S. Chereskin

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[57] ABSTRACT

There is provided a method for transferring Tripsacum nuclear and cytoplasmic genes into maize. The method is via a hybrid plant designated Tripsacorn (proposed botanical classification *Zea indiana*), produced by crossing two wild relatives of corn, Tripsacura and diploid perennial teosinte (*Zea diploperennis*). This invention thus relates to the hybrid seed, the hybrid plant produced by the seed and/or tissue culture, variants, routants, and modifications of Tripsacorn and the hybrid seed, the hybrid plant produced by the seed and/or tissue culture, variants, mutants, and modifications of (maize X Tripsacorn) and/or (Tripsacorn X maize). In particular this invention is directed to the ability to confer rootworm resistance, resistance to insect pests, resistance to diseases, drought tolerance, and improved standability to maize via Tripsacorn.

12 Claims, No Drawings

METHODS AND MATERIALS FOR CONFERRING TRIPSACUM GENES IN MAIZE

This application is a continuation-in-part of application Ser. No. 07/613,269, filed Nov. 13, 1990, issued as U.S. Pat. No. Plant 7,977 on Sep. 15, 1992 the disclosure of which is incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates generally to the field of plant breeding. More particularly, it relates to a method for the production of inbred and hybrid corn with desirable characteristics including corn rootworm resistance, resistance to insect pests, resistance to diseases, drought tolerance and improved standability conferred by Tripsacum introgression via a bridge species called Tripsacorn.

BACKGROUND OF THE INVENTION

Plant breeding is the science that utilizes crosses between individuals with different genetic constitutions. The resulting recombination of genes between different lines, species or genera produces new hybrids from which desirable traits are selected. Methods employed to develop new varieties or species depend on whether a crop plant reproduces sexually or asexually. Since maize is a sexually reproducing plant, techniques for controlled pollination are frequently employed to obtain new hybrids.

A significant technological breakthrough in maize breeding was the discovery that crossing inbred lines resulted in a hybrid with greatly enhanced vigor. Inbred lines are obtained from self-pollination and selection of homozygous plants for several generations until a pure line descended by self-pollination from an apparently true-breeding plant is obtained. The purpose of inbreeding is to fix desirable characters in a homozygous condition in order that the line may be maintained without genetic change. Inbred lines with desired traits are then crossed to produce commercial hybrids. Yields from hybrid maize seed are much greater than average yields of inbreds and open-pollinated varieties.

Maize is a monoecious grass, i.e. it has separate male and female flowers. The staminate, i.e. pollen-producing, flowers are produced in the tassel and the pistillate or female flowers are produced on the shoot. Pollination is accomplished by the transfer of pollen from the tassel to the silks. Since maize is naturally cross-pollinated, controlled pollination, in which pollen collected from the tassel of one plant is transferred by hand to the silks of another plant, is a technique used in maize breeding. The steps involved in making controlled crosses and self-pollinations in maize are as follows: (1) the ear emerging from the leaf shoot is covered with an ear shoot bag one or two days before the silks emerge to prevent pollination; (2) on the day before making a pollination, the ear shoot bag is removed momentarily to cut back the silks, then is immediately placed back over the ear; (3) on the day before making a pollination, the tassel is covered with a tassel bag to collect pollen; (3) on the day of pollination, the tassel bag with the desired pollen is carried to the plant for crossing, the ear shoot bag is removed and the pollen dusted on the silk brush, the tassel bag is then immediately fastened in place over the shoot to protect the developing ear. Wild relatives of crop plants are an important source of genetic diversity and genes well adapted to many different stresses. The wild relatives of maize include annual teosinte (*Zea mexicana*), perennial teosinte and Tripsacum. *Zea diploperennis* (hereafter referred to as diploperennis), is a diploid perennial teosinte. A previously unknown wild relative of maize, it was discovered on the verge of extinction in the mountains of Jalisco, Mexico in 1979. Diploperennis, like annual teosinte, is in the same genus as maize, has the same chromosome number (n=10), and hybridizes naturally with it. Tripsacum is a more distant relative of maize with a different haploid chromosome number (n=18). The progeny of (maize X Tripsacum) obtained by artificial methods are all male sterile and have limited female fertility when pollinated by maize pollen. Cytogenetic studies of maize-Tripsacum hybrids show partial chromosome pairing and homology between segments of Tripsacum and maize chromosomes (Maguire 1961, 1963; Chaganti 1965; Gallnat 1974). In spite of strong cross-incompatibility, the fact that maize and Tripsacum chromosomes can occasionally pair enables limited transfer of Tripsacum genes into maize. Attempts to make the corollary cross, i.e. between Tripsacum and teosinte, however, have heretofore failed to produce viable plants (Tantravahi 1968; deWet and Harlan 1978).

Plant breeders acknowledge Tripsacum has significant potential for improving corn by expanding its genetic diversity (Gallnat 1977; Cohen and Galinat 1984; Poehlman 1986). The limited fertility of maize-Tripsacum hybrids presents a significant biological barrier to gene flow between these species. Successful introgression of Tripsacum genetic material into maize heretofore has required years of complicated, high risk breeding programs that involve many backcross generations to stabilize desirable Tripsacum genes in maize. According to Kindiger and Beckett: "Tripsacum may be expected to contain valuable agronomic characters that could be exploited for the overall improvement of maize . . . An effective procedure to transfer Tripsacum germ plasm into maize has been needed by maize breeders and geneticists for many years" (1990, p. 495). Beneficial traits that may be derived from Tripsacum include heat and drought tolerance (Reeves and Bockholt 1964), elements of apomixis, increased heterosis (Reeves and Bockholt 1964; Cohen and Galinat 1984), resistance to corn root worm (Branson 1971), corn leaf aphid (Branson 1972), northern and southern leaf blight, common rust, anthracnose, fusarium stalk rot and Stewart's bacterial blight (Bergquist 1977, 1981; deWet 1979).

(*Zea mays X Tripsacum*) plants have unreduced gametes with 28 chromosomes, one set of 10 Zea chromosomes and one set of 18 Tripsacum chromosomes. There has been one report of a successful reciprocal cross of Tripsacum pollinated by maize in which embryo culture techniques were used to bring the embryo to maturity. The plants were sterile (Farquharson 1957). This (*Tripsacura* X maize) plant was employed by Branson and Guss (1972) in tests for rootworm resistance in maize-*Tripsacum* hybrids. When the (maize X Tripsacura) hybrid has been crossed with either annual teosinte or diploperennis, a trigenomic hybrid has been produced that has a total of 38 chromosomes; 10 from maize, 18 from Tripsacum and 10 from teosinte. The resulting trigenomic plants were all male sterile and had a high degree of female infertility (Mangelsdorf 1974; Galinat 1986).

Transformation, a technique from molecular biology, now offers opportunity for the asexual transfer of genes that heretofore could only be achieved by crossing

different plant strains. In order for breeders to employ gene transfer via transformation, they first have to be able to achieve plant regeneration from calli or protoplasts. Although transformation has been successfully performed in maize (Gordan-Kamm et al. 1990), there is limitation in developing transgenic maize due to the difficulties of plant regeneration from maize protoplasts (Potrykus 1990). The problem is there are very few maize lines that can be successfully regenerated from maize protoplasts. In order for transformation to be useful for commercial hybrid seed production, it will be necessary to have inbred lines amenable to the transgenic process that can be regenerated by tissue culture.

Rootworms, Diabrotica spp., are a serious agricultural pest. Reduction in corn yields due to corn rootworm damage ranges from 13 to 16 bushels per acre which is approximately 10 to 13%. Costs of insecticide treatments and crop losses are estimated at $1 billion per year (Metcalfe 1986). Rootworm larvae feed on the root system of corn for several weeks passing through three instars. This is the most destructive stage and causes reduced yields through damage to the root system or indirectly from lodging which makes plants difficult to harvest. Adult beetles feed on the aerial parts of the corn plant including the pollen, silks and leaves (Branson et al. 1975).

Zea diploperennis is an acceptable larval host for several Diabrotica species. Feeding scars and leaf damage have been recorded for plants growing in the wild in Jalisco, Mexico, and laboratory screening revealed diploperennis has no antibiotic effect on rootworm larvae (Branson and Reyes 1983). *Tripsacum dactyloides*, however, has been shown to exhibit a high degree of resistance to corn rootworm (Branson 1971). Screening of intergeneric hybrids between *T. dactyloides* and *Zea mays* showed (maize X *Tripsacuraum*) was susceptible; whereas, (*Tripsacura* X maize) exhibited resistance (Branson and Guss 1972). The authors proposed two explanations: (1) resistance is inherited through the cytoplasm, or (2) the genes for resistance occur on lost *Tripsacum chromosomes* in (maize X *Tripsacum*) plants.

Polyploidy refers to all natural and induced variations in chromosome number. Many cultivated crop species have evolved in nature as polyploids. One way polyploid plants arise is by combining chromosome sets from two or more species which is referred to as allopolyploidy. An allopolyploid, i.e. a plant in which the total chromosome complement of two other species is combined to form a fertile species hybrid, is referred to as an amphiploid. A plant breeding method to transfer genes across a barrier of reproduction isolation is via bridging species derived from an amphiploid. This type of introgressive hybridization produces convergence between previously more distinct species. It may result in the appearance of types that are new species intermediate to their more divergent and distinct parents. Bridging species derived from crosses between two parents with different chromosome numbers are frequently characterized by a new chromosome number. The change in chromosome complement and/or rearrangements in chromosome structure may overcome the inability of chromosomes to pair that causes infertility and often prevents the success of wide crosses.

Two wild grasses, *Zea diploperennis* and *Tripsacum dactyloides* have been crossed to produce a novel hybrid referred to as Tripsacorn, proposed botanical name *Zea indiana*. A bridging mechanism to transfer *Tripsacum genes* into maize is provided by Tripsacorn which is cross-fertile with maize. It promises to improve corn by imparting numerous beneficial characteristics including pest resistance and drought tolerance.

Based on proposed taxonomic relationships between Zea and Tripsacura and the results of prior crosses between them, the success of the crosses between *Zea diploperennis* and *Tripsacura* resulting in fully fertile plants with chromosome numbers of 2n=20 and 2n=18 could not have been predicted. The reduction in chromosome number in the interspecific cross is unexpected based on prior art. The fertility of plants resulting from the cross made both ways is also unexpected. Tripsacum and diploperennis have chromosomes that are very similar architecturally in length and their diminutive, terminal knobs that appear at one or both ends of many of the chromosomes in both species. The small terminal knobs in these species are distinct from the large internal knobs that characterize the chromosomes of corn and annual teosinte. As evidenced by cross fertility and chromosome number, the similarities in the chromosome structure of Tripsacum and diploperennis evidently promote a greater degree of pairing and enable the unexpected success of this cross.

The unexpected fertility of this hybrid, and its cross-fertility with maize, is of great value because it conveys opportunity for directly crossing with maize. Tripsacorn provides a mechanism for importing Tripsacum genes into maize in one generation by natural breeding techniques. Since Tripsacum is the female parent in this cross, it provides unique opportunity for transferring Tripsacum cytoplasmic genes into maize.

Insect resistance derived from crossing Tripsacorn with maize has been demonstrated experimentally. In a series of bioassays, seedlings from (maize X Tripsacorn) infested with western corn rootworm, *Diabrotica virgifera* Le Conte, showed clear evidence for rootworm resistance. This was corroborated by comparison with maize controls and (maize X Sun Dance) plants, both of which were susceptible, as indicated by considerable root damage or death.

REFERENCES

Bergquist, R. R. 1977. Techniques for evaluation of genetic resistance in corn. North Central Regional Corn and Sorghum Disease Project, Chicago.

Bergquist, R. R. 1981. Transfer from *Tripsacum dactyloides* to corn of a major gene locus conditioning resistance to *Puccinia sorghi*. Phytopathology 71:518–520.

Branson, T. F. 1971. Resistance in the grass tribe Maydeae to larvae of the western corn rootworm. Ann. Ent. Soc. Am. 64:861–863.

Branson, T. F. 1972. Resistance to the corn leaf aphid in the grass tribe Maydeae. J. Econ. Entomol. 65:195–196.

Branson, T. F. 1986. Larval feeding behavior and host-plant resistance in maize. In J. L. Krysan and T. A. Miller (ed.) Methods for the Study of Pest Diabrotica. Springer Verlag, New York.

Branson, T. F. and P. L. Guss. 1972. Potential for utilizing resistance from relatives of cultivated crops. Proc. North Central Branch Entomol. Soc. Am. 27:91–95.

Branson, T. F., P. L. Guss, J. L. Krysan and G. R. Sutter. 1975. Corn rootworms: Laboratory rearing and manipulation. U.S. Dept. Agric., ARS-NC-28. 18 pp.

Branson, T. F. and J. Reyes R. 1983. The association of Diabrotica spp. with Zea diploperennis. J. Kan. Entomol. Soc. 56:97-99.

Chaganti, R. S. K. 1965. Cytogenetic studies of maize-Tripsacum hybrids and their derivatives. Harvard Univ. Bussey Inst., Cambridge, Ma.

Cohen, J. I. and W. C. Galinat. 1984. Potential use of alien germplasm for maize improvement. Crop Sci. 24:1011-1015.

DeWet, J. M. J. 1979. Tripsacum introgression and agronomic fitness in maize (Zea mays L.). Proc. Conf. Broadening Genet. Base Crops, Puduc, Wageningen.

DeWet, J. M. J. and J. R. Harlan. 1978. Tripsacum and the origin of maize. In D. B. Walden (ed.) Maize Breeding and Genetics, John Wiley & Sons, New York.

Elseman, L. and L. Herbert, 1990. *The Pantone Book of Color.* Harry N. Abrams, Inc., Publishers, New York.

Farquharson, L. I. 1957. Hybridization of Tripsacum and *Zea. J. Heredity* 48: 295-299.

Galinat, W. C. 1974. Intergenomic mapping of maize, teosinte and Tripsacura. Evolution 27: 644-655.

Galinat, W. C. 1977. The origin of corn. In G. F. Sprague (ed.). Corn and Corn Improvement. Amer. Soc. Agronomy, Madison, Wis.

Galinat, W. C. 1982. Maize breeding and its raw material. In W. L. Sheridan (ed.) Maize for Biological Research. University Press, Grand Forks, N.D.

Galinat, W. C. 1986. The cytology of the trigenomic hybrid. Maize Genetics Newsletter 60:133.

Gordon-Kamm, W. 1990. Transformation of maize cells and regeneration of fertile transgenic plants. Plant Cell 2:603.

Hills, T. M. and D. C. Peters. 1971. A method of evaluating postplanting insecticide treatments for control of western corn rootworm larvae. J. Econ. Entomol. 64: 764-765.

Iltis, H. H., J. Doebley, R. Guzman M. and B. Pazy, 1979. *Zea diploperennis* (Gramineae): A new teosinte from Mexico. Science 203: 186-188.

Kindiger, B. and J. B. Beckett. 1990. Cytological evidence supporting a procedure for directing and enhancing pairing between maize and Tripsacura. Genome 33: 495-500.

Maguire, M. P. 1961. Divergence in Tripsacura and Zea chromosomes. Evolution 15: 393-400.

Maguire, M. P. 1963. Chromatid interchange in allodiploid maize-Tripsacum hybrids. Can. J. Genet. Cytol. 5:414-420.

Mangelsdorf, P. C. 1974. Corn: Its origin, evolution and improvement. Harvard Univ. Press, Cambridge, Ma.

Metcalfe, R. L. 1986. Foreword. pp.vii-xv. In J. L. Krysan and T. A. Miller (ed.) Methods for the Study of Pest Diabrotica. Springer Verlag, New York.

Poehlman, J. M. 1986. Breeding Field Crops. 3rd ed. AVI Publ. Co., Inc., Westport, Conn.

Potrykus, I. 1990. Gene transfer to cereals: An assessment. Biotechnology:535-542.

Reeves, R. G. and A. J. Bockholt. 1964. Modification and improvement of a maize inbred by crossing it with Tripsacum. Crop Sci. 4:7-10.

Tantravahi, 1968. Cytology and crossability relationships of Tripsacum. Harvard Univ. Bussey Inst., Cambridge, Ma.

SUMMARY OF THE INVENTION

In one embodiment of the invention, there is provided a method for conferring Tripsacum nuclear and cytoplasmic genes in maize. In the first step of the method, *Tripsacura dactyloides* (female) is crossed by *Zea diploperennis* (male) by controlled pollination technique. The resulting intergeneric hybrid derived in step 1 is fully fertile and cross-fertile with maize. It is characterized by its utility as a genetic bridge to transfer Tripsacura genes into corn.

In another embodiment of the invention, the intergeneric (*Tripsacum dactyloides X Zea diploperennis*) hybrid plant derived from step 1, referred to as Tripsacorn, and maize are crossed by controlled pollination. This invention relates to the hybrid seed, the hybrid plant produced by the seed and/or tissue culture, variants, mutants, and modifications of Tripsacorn, of (maize X Tripsacorn) and of (Tripsacorn X maize).

In another embodiment of the invention, there is provided plants and plant tissues produced by the method of crossing maize (female) by Tripsacorn (male) which exhibit resistance to rootworm.

In another embodiment of the invention, there is provided plants and plant tissues produced by the method of crossing maize (female) by Tripsacorn (male) which exhibit tolerance to drought.

In another embodiment of the invention, there is provided plants and plant tissues produced by the method of crossing maize (female) by Tripsacorn (male) which exhibit enhanced resistance to disease.

In another embodiment of the invention, there is provided plants and plant tissues produced by the method of crossing maize (female) by Tripsacorn (male) which exhibit enhanced resistance to insect pests.

In another embodiment of the invention, there is provided plants and plant tissues produced by the method of crossing maize (female) by Tripsacorn (male) which exhibit resistance to lodging.

For the purposes of this application, the following terms are defined to provide a clear and consistent description of the invention.

Allopolyploid. An individual with two or more chromosome sets.

Amphiploid. An individual with two or more genomes derived from different species.

Antibiosis. Antibiosis refers to the plant's ability to adversely effect the insect pest, for example by producing a toxic substance.

Antixenosis. Antixenosis refers to the plant's ability to detract the insect pest away from the plant, for example by producing a deterrent substance.

Hybrid plant. An individual plant produced by crossing two parents of different genotypes.

Polyploid. An individual with some variation in normal diploid chromosome number.

Root Lodging. Root lodging is indicated when plants lean from the verticle axis an at angle $\geqq 30°$.

Tolerance. Tolerance is indicated when a plant sustains rootworm damage but is still able to grow in spite of damage.

DETAILED DESCRIPTION OF INVENTION

The method of the invention is performed by crossing *Tripsacum dactyloides* and *Zea diploperennis.* The crosses are performed using standard plant breeding techniques for controlled pollinations known in the art.

Thus, the present invention provides a method of producing hybrid plant seeds comprising the steps of (a) crossing a Tripsacura species (e.g. *Tripsacura dactyloides*) female parent with a Zea species (e.g. *Zea diploperennis*) male parent to produce seed; then (b) harvesting the seed produced.

This method produces a hybrid seed and a hybrid plant, from which tissue cultures can be made. Additionally, pollen produced by the hybrid plant can be collected.

The term "plant" as used in this application refers to the whole plant as well as its component parts, e.g., flowers, roots, fruits, and rhizomes.

The present invention further provides a method of producing hybrid corn seed comprising the steps of (a) crossing a *Tripsacura dactyloides* female parent with a *Zea diploperennis* male parent to produce (*Tripsacura dactyloides X Zea diploperennis*) hybrid seed; then (b) growing a (*Tripsacura dactyloides X Zea diploperennis*) hybrid plant from said seed to maturity; then (c) crossing said (*Tripsacura dactyloides X Zea diploperennis*) hybrid plant with maize to produce seed and (d) harvesting the seed produced.

This method results in the production of hybrid corn seed and hybrid corn plants, from which tissue cultures can be made. One marked benefit of the present invention is the production of hybrid corn plants which exhibit enhanced resistance to corn rootworm.

Plant breeding techniques and tissue culture techniques as described herein are known, and may be carried out in the manner known to those skilled in the art. See, for example, U.S. Pat. No. 4,737,596 to Seifert et al. entitled "Hybrid Corn Plant and Seed"; U.S. Pat. No. 5,059,745 to Foley entitled "Hybrid Corn Line LH195"; U.S. Pat. No. 4,545,146 to Davis entitled "Route to Hybrid Soybean Production"; U.S. Pat. No. 4,627,192 to Fick entitled "Sunflower Products and Methods for their Production", and U.S. Pat. Nos. 4,837,152 and 4,684,612 entitled "Process for Regenerating Soybeans" Applicant specifically intends that the disclosure of all U.S. patent applications cited herein be incorporated herein by reference.

In Tripsacum inflorescences, the staminate (i.e. male) flowers and pistillate (i.e. female) flowers are produced on a single spike with the male flowers subtended by the female. When Tripsacum sends out the inflorescence, the staminate flowers are broken off leaving only the female flowers on the spike which is then covered with a pollinating bag, i.e. standard ear shoot bag for maize, to protect them from contamination by unwanted pollen. Diploperennis male and female flowers occur on separate parts of the plant. The staminate flowers are borne in the tassel which emerges at the apex of the culm; whereas, the pistillate flowers occur in single-rowed spikes borne on lateral branches of the culm. When diploperennis produces its tassels, they are covered with a pollinating bag. When they start shedding pollen, the bag is removed and pollen taken to pollinate the Tripsacum plants. At that time, the bags covering the Tripsacum pistillate flowers are removed and the diploperennis pollen shaken out of the bag onto the silks. The Tripsacum inflorescence is covered again with a pollinating bag immediately after pollination and the bag is stapled so that it remains on the spike until the seed has matured. Upon maturity, approximately 45 days later, the seed is harvested. Once mature seed from the cross has been obtained, it is germinated on moist filter paper in a petri dish in the dark. When the seed starts to germinate, it is transferred to potting soil in a pot. The plants are grown in the greenhouse or outdoors. Controlled crosses are best made in a greenhouse where plants are kept isolated to prevent cross contamination and there is no problem with bags being damaged by weather conditions.

This method may alternatively be used to cross the plants with diploperennis as the female parent. In this embodiment, all the tassels, i.e. male flowers, are removed from the diploperennis plant as soon as they emerge and the ears, i.e. female flowers, are covered with pollinating bags. Rather than removing Tripsacum male flowers, the spikes are left intact and covered with a pollinating bag to collect Tripsacum pollen. The pollen is applied to the diploperennis ears which are then immediately covered with a pollinating bag that is well fastened with staples to ensure it remains sealed until the seed has matured, approximately 45 days after pollination when the seed is harvested.

Next, when (*Tripsacum X diploperennis*) starts to flower, the same steps described above are used to cross the hybrid with maize. To cross onto maize, as soon as the maize plants begin to produce ears, before the silks emerge, the ears are covered with an ear shoot bag. Pollen collected from (Tripsacum X diploperennis) is applied to silks of the maize ears. The ears are then covered again with an ear shoot bag and a large pollinating bag which is wrapped around the culm and secured with a staple. The ears remain covered until they reach maturity, several weeks later when the ears are harvested.

Plants grown from all crosses described above are male and female fertile and are cross-fertile with each other.

The principles and techniques used in breeding insect and disease resistance are basically the same. First, sources of resistance genes must be located. Secondly, genes for resistance must be transferred into adapted varieties by hybridization procedures, and thirdly, those varieties must be exposed to the insect pest or disease under natural or artificially induced conditions in order to distinguish resistant strains from susceptible strains. The mode of inheritance of resistance may be simple and involve only one to two major genes. Though in most cases resistance is dominant, it may be dominant or recessive. Inheritance of resistance also may be more complex with numerous genes affecting the host-parasite relationship. Plant breeders test for resistance by experimental inoculation of plants grown in the field and/or the greenhouse. In testing for rootworm resistance, artificially reared insects are transferred to plants grown in the field or a greenhouse, or to newly germinated seedlings in petri dishes. The infected plants are observed and evaluated according to specific criteria for a particular pest. In looking for rootworm resistance, criteria for evaluation include observations of plant lodging and scoring of root damage by a standardized scale.

Tripsacum X diploperennis

A detailed description of the plants obtained from (*Tripsacum X diploperennis*) is outlined below.

Origin: Seedling

Parentage:

Seed parent.-*Tripsacum dactyloides*

Source: Established clone at Indiana University, Bloomington, Ind.

Pollen partent.-*Zer diploperennis*

Source: Jalisco, Mexico (Reference Iltis et al., 1979)

Classification: Botanic—*Zea indiana* (proposed).

Cytology: Diploid chromosome number determined from root tips ranged from 2n=18 to 2n=20.

Habit: Essentially erect; as many as 35 primary culms, usual number about 15.

Duration:

Perennial.—Sends out shoots from rhizomes. Plant will freeze at winter temperatures below 28° F., but new growth is produced in spring after winter temperatures of 0° F. Culm:

Height.—Up to two meters: slender, simple with occasional branching from the nodes of the culm; glabrous; oval in cross section; diameter 1–1.2 cm.

Nodes.—glabrous, aerial roots develop at nodes along culm.

Sheath.—tightly closed enwrapping the culm, margins not united; glabrous;turns rose red (Pantone #18-1852) when exposed to sun, otherwise green; rose red (Pantone #18-1852, ciliate auricles at summit margins.

Ligule.—present on adaxial side of leaf at junction of blade and sheath; length: 4 nun; membranaceous, irregular edge.

Leaf blade: Alternate; distichous; sheathing base; parallel veined; narrowly linear, flat, thin.

Length.—47–56 cm. Width: 1.5–5.0 cm.

Entire margin.—Rose red (Pantone #18-1852), serrulate.

Midrib.—White (Pantone #12-5202).

Adaxial surface.—Sparsely hirsute.

Abaxial surface.—Glabrous except sparsely hirsute along midrib.

Prominent parallel veins.—5 per 1 cm width.

Inflorescence

Blooming period: Twice annually in the greenhouse for approximately one month beginning in late April and late October in Tennessee, North Carolina and Mississippi.

Monoecious: Separate male and female flowers on the same plant; variable.

Staminate flowers: May be of two types: one inflorescence type borne as paired spikelets on a slender rachis forming 3–7 racemes arranged in a panicle, the "tassel", at the summit of the culm. Alternatively, staminate spikelets may be borne on a single spike above the pistillate flowers.

Length.—6–12 cm.

Axis.—stiff, continuous, ascending.

Spikelet: Two-flowered, one sessile, one pedicled; laterally compressed awnless, attenuate with red (Pantone #19-1860) tip and red (Pantone #19-1860) band at base; Length: 11 mm;

Width: 3 mm. In pairs on one side of a persistent central axis.

Pedicel length.—3 mm.

Glumes.—Outer glume: cartilaginous, tapering to an acute tip, ciliate, flat, several nerved, margins involute, fimbriate.

Inner glume: chartaceous.

Pistillate flowers: Borne in leaf axils; spikelets distichously arranged; variable.

Styles: pilose with distinct bifurcated tips.

Color: Ranges from pastel parchment (Pantone #11-0603) to light lilac (Pantone #12-2903) to rose red (Pantone #18-1852).

Length: 100 mm.

One type of pistillate flower consists of a single rowed spike of 4 to 6 triangular caryopses in hard, shell-like fruitcases enclosed in a single leaf sheath; caryopses disarticulate upon maturity.

Length: 7.5 mm; Width: 5 mm.

Colors range from solid to variegated combinations of the following:White (Pantone #11-0602), gray (Pantone #16-1107) , tobacco brown (Pantone #17-1327), brown (Pantone #19-1121) , dark brown (Pantone #19-1020).

Alternatively, spikelets paired and partially enclosed in stiff, brown speckled glumes; caryopses rounded and imbricate; Spikes enclosed in single or multiple leaf sheaths. Caryopses do not disarticulate upon maturity;

Length: 5 mm; Width: 5 mm.

Color variegated combinations of the following: dark brown (Pantone #19-1217, brown (Pantone #18-1154), beige (Pantone 15-1225), light beige (Pantone #13-1018).

Fruit: Five to ten ears per culm per blooming period; flowers are produced twice a year under greenhouse conditions; some plants may produce approximately 150 ears twice annually.

Maturity: 45 days following fertilization.

Ear (Husked Ear Data Unless Stated Otherwise)

Length: About 43 mm.

Midpoint diameter: About 6.7 mm.

Weight: 0.5 gm.

Kernel rows: 2 (rarely 3–4)

Silk color (exposed at silking stage): light lilac (Pantone #12-2903) to rose red (Pantone #18-1852).

Husked color: Cob kernels are embedded in the rachis segments, some of which disarticulate upon maturity. These segments are brownish gray and are the hard, bony fruitcases enclosing the kernels.

Kernel color: beige (Pantone #14-1122) shading to golden beige (Pantone #16-1336).

Husked extension (harvest stage): About 1 cm.

Shank: About 6.5 cm.

Taper: Slight.

Position in dry husk stage: Upright.

Drying time (unhusked ear): About 2–3 days.

Kernel (Dried)

Type I: Angular caryopses in hard, shell-like fruitcases, disarticulate upon maturity:

Size (from midpoint): Length about 0.8 mm, width about 0.5 mm, thickness about 0.4 mm.

Shape: Trapezoidal

Colors range from solid to variegated combinations of the following: white (Pantone #11-0602), gray (Pantone #16-1107), tobacco brown (Pantone #17-1327), brown (Pantone #19-1121) , dark brown (Pantone #19-1020)

Weight 20 seeds (unsized samples): 1.3 gm.

Type II: Paired caryopses partially enclosed in endurated glumes forming a cob, upon maturity do not disarticulate:

Size (from midpoint): Length about 3.9 mm, width about 2.8 mm, thickness about 2.7 mm.

Shape grade (% round): 100% round (tip pointed).

Pericarp color: beige (Pantone #14-1122) shading to

golden beige (Pantone #16-1336).

Aleurone color: Clear.

Endosperm color: White (Pantone #11-0601).

Endosperm type: Pop.

Weight 20 seeds (unsized samples): About 0.4 gin.

Cob
Diameter at midpoint: 5.3 to 8.7 mm.
Strength: Variable.
Color: Smoke (Pantone #12-0704).
Alicole: Length: About 6.6 mm. External width: 7.0 mm. Internal width: 5.0 mm. External length: 5.5 mm. Internal length: 5.0 mm. Thickness: Approximately 4.5 mm. Depth: 2.9 mm.
Cupule: Overhang: About 0.6 mm. Wing height: 4.1 mm. Left wing width: 1.0 mm. Right wing width: 1.3 mm. Lower glume length: 5.9 mm. Lower glume width: ~3.0 mm. Lower glume angle: ~20°Glume cushion width: 5.4 mm. Glume cushion height: 1.8 mm. Sessile thickness: 0.3 mm. Cupule pubescence: sparse, short hairs. Color: Buff (Pantone #13-1024).

Comparative Parental Characteristics

Duration: *Z. diploperennis* does not survive temperatures below approximately 24° F. *T. dactyloides* is a true perennial and produces new growth every year surviving temperatures are below 0° F.
Leaf blade: *Zea diploperennis* round in cross section; diam. 1 cm. *Tripsacum dactyloides* oval in cross section; diam. 1.3 cm.
Leaf blade: *Z. diploperennis.* Width 1-2 cm; margins pink serrulate from midsection of blade to tip; adaxial surface: sparsely hirsute; prominent veins: 6 per 1 cm width. *T. dactyloides.* Width: 1 cm; margins white serrulate along entire blade; Adaxial surface: hirsute; prominent veins: 12 per 1 cm.
Blooming period: *Z. diploperennis* twice a year in the greenhouse, end of March and end of September for about a month. *T. dactyloides* continuously from May to October.
Staminate flowers: *Z. diploperennis* borne in tassel at summit of culm. *T. dactyloides* staminate flowers borne above pistillate flowers in single spike.
Pistillate flowers: *Z. diploperennis* caryopsis triangular-trapezoidal in hard bony fruitcases; Length: 8 mm; Width: 4–5 mm; Color: black (Pantone #19-0303), dark brown (Pantone #19-1020) or mottled black-brown. *T. dactyloides* caryopsis trapezoidal in hard, bony fruitcase; Length: 6–10 mm; Width: 6 mm. Color: pale brown (Pantone #17-1137) or buff (Pantone #13-1024).

Maize X Tripsacorn

(Maize X Tripsacorn) plants look basically like maize. One difference when comparing these plants to maize controls is that they are shorter, have stronger stalks and are not susceptible to lodging. The ears look like maize and are equal in weight to maize ears. However, the kernels tend to be larger in size than kernels of maize controls. The plants produced by (maize X Tripsacorn) do not show as many signs of infestation by insects or disease as maize controls. Noticeable resistance to aphids and white flies has been observed on plants grown in the greenhouse and enhanced resistance to corn earworm and ear and kernel rot has been observed in plants grown in the field. Laboratory bioassays have shown enhanced resistance to corn rootworm. When subjected to dry conditions, (maize X Tripsacorn) plants do not exhibit signs of wilting and drought stress to the same extent as maize controls.

EXAMPLES

Bioassays for Determining Rootworm Resistance in Maize

Two types of bioassays, in petri dishes and in pots, were conducted to determine if Tripsacorn could impart rootworm resistance to maize. For the bioassays, 1,000 non-diapausing western corn rootworm eggs in soil were shipped from French Agricultural Research, Inc., Lamberton, Minn., to Durham, N.C., under U.S. Department of Agriculture permit number 922762. Plants were infested with newly hatched first instar larvae of western corn rootworm, *Diabrotica virgifera.* The larvae were transferred to test containers by lifting with a small paint brush. Two separate petri dish bioassays and three pot bioassays were performed.

For the bioassays, seed from Tripsacorn crossed to four diverse types of maize was used. The four types included: a commercial hybrid corn seed Funk's G4522; two inbred lines, B73 and W64A; a native Mexican race, Zapalote Chico, classified as a prehistoric mestizo indicating derivation from ancient indigenous races. Other plants infested with corn rootworm included (G4522 X Sun Dance), Tripsacum, Tripsacorn and maize controls.

Petri Dish Bioassays

Petri dish bioassays were employed to screen for antibiosis versus antixenosis by observing whether larvae remained on the roots, ate them and survived or died; or whether larvae moved away from the roots. If there is an antibiotic effect, evidence for eating and dead larvae can be seen; if there is an antixenotic effect, larvae can be observed trying to leave the dish. For these tests, 10 grams of top soil sieved through a 1 mm mesh screen was placed in a petri dish with 3 to 5 freshly germinated seedlings or, in the case of Tripsacum, with a small clonal piece of plant with young roots, and kept moist. The rims of each dish were ringed with petroleum jelly to monitor for any larvae trying to leave the dish. Up to a total of 50 larvae were added to each dish over a three day period. Each treated dish was observed for several days under a dissecting microscope at 60X magnification and behavior recorded.

The plants used in the petri dish bioassays and observed results are summarized in Table I. In all cases, larvae remained on or near the roots, seed and cotyledons or in the soil. There was no indication of larvae trying to exit the petri dishes and thus, it is concluded no evidence for antixenosis. Tripsacum, Tripsacorn, (B73 X Tripsacorn), (W64A X Tripsacorn), (G4522 X Tripsacorn) and (G4522 X Sun Dance) did not show any signs that the roots produce a substance that is a deterrent to the insects. Larvae feeding was observed in all cases and severity of root damage rated by the Hills and Peters scale. Evidence for antibiosis and tolerance was indicated with Tripsacorn and the hybrids between corn and Tripsacorn tested; whereas, there was no evidence for antibiosis or tolerance with the corn and (maize X Sun Dance) materials tested.

TABLE I

RESULTS OF PETRI DISH BIOASSAYS

	No. of Larvae	Observations/Comments
Bioassay #1		
Tripsacum	50	Larvae stay on root, some feeding but virtually no damage to roots. larvae not visible

TABLE I-continued

RESULTS OF PETRI DISH BIOASSAYS		
	No. of Larvae	Observations/Comments
		after a couple of days
Tripsacorn	50	Some feeding, little root damage
B73 X Tripsacorn	50	Some feeding, little root damage, plants continue to grow
G4522 X Tripsacorn	50	Some feeding, little root damage, plants continue to grow
G4533 X Sun Dance	50	Extensive feeding, plants died
Corn control	50	Extensive feeding, plants died
Bioassay #2		
Tripsacorn	20	Light feeding, some dead larvae
Corn control (W64A)	45	Extensive feeding, plants died
W64A X Tripsacorn	45	Feeding on roots, seed and cotyledons, some dead larvae

Pot Bioassays

Plants grown in pots were used to screen for evidence of tolerance and/or antibiosis. Lodging is seen in plants that are susceptible to rootworm damage; whereas, plants that remain upright and healthy when exposed to rootworms are indicative of tolerance and antibiosis. Root damage was observed and scored according to the Hills and Peters (1971) rating scale of 1-6 that is widely used in the corn belt to evaluate root damage. The criteria for rating are as follows:

1. No damage or only a few minor feeding scars
2. Feeding scars evident but no roots eaten off to within 1 ½ inch of the plant
3. Several roots eaten off to within 1½ inch of the plant but never the equivalent of an entire node of roots destroyed
4. One root node completely destroyed
5. Two root nodes completely destroyed
6. Three or more root nodes destroyed

When a bioassay was complete, two to three plants were removed from the pots, soaked in water then rinsed with a gentle water spray to clean the roots, then observed under a dissecting microscope for scoring. The score reported is the mean calculated from the total scores of plants in each category. Tolerant plants may suffer root damage but are capable of regrowth and degrees of plant recovery. Well developed secondary root systems are often capable of compensatory growth from damaged crown roots.

In the first pot bioassay, 3 to 5 seedlings (approximately 1 week old), or in the case of Tripsacum a small clone with young roots, were planted in potting soil in 10-ounce containers and were grown indoors under artificial grow lights. A total of 70 larvae were added to each container over a two day period and plants were observed for 11 days.

In the second pot bioassay, ≦10 day old seedlings were planted in potting soil in 3 inch peat pots and grown indoors under artificial grow lights. A total of 30 larvae were added to each pot over a three day period. Although most plants were dead within one week, observation of the ones that survived extended over two weeks before plants were sacrificed for root evaluation. For each type, there were a minimum of two plants, and in most cases there were four plants.

In the third pot bioassay, the plants were 11 to 14 days old at infestation and they were grown on a porch under natural sunlight. A total of 30 larvae were added to each pot over two days. They were observed for 11 days before sacrificing plants to score root damage.

The plants used in the pot bioassays and observed results are summarized in Table II. The results indicate that (maize X Tripsacorn) plants are definitely more resistant to corn rootworm than corn controls and (maize X Sun Danse). The mechanisms indicated for resistance inherited from Tripsacorn are antibiosis and tolerance. All the plants sustained some injury to the roots. Lodging in the corn controls and (maize X Sun Dance) plants was ≧45° and rating on the Hills and Peters scale ranged from 5 to 6. The corn X Tripsacorn plants remained upright and appeared healthy, but did sustain root damage. There was good development of secondary roots from the damaged crown showing the capability for compensatory growth in all the (maize X Tripsacorn) plants.

TABLE II

RESULTS OF POT BIOASSAYS				
	No. of Larvae	Duration	Root Damage*	Observations/Comments
Bioassay #1				
Tripsacum	70	11 days	Not recorded	No sign of damage
Tripsacorn	70	11 days	Not recorded	No sign of damage
B73 X Tripsacorn	70	11 days	Not recorded	No sign of damage
G4522 X Tripsacorn	70	11 days	Not recorded	No sign of damage
G4522 X Sun Dance	70	11 days	Not recorded	Plants died after 6 days
Bioassay #2				
Corn control (W64A)	30	14 days	5.0	Plants died
W64A X Tripsacorn	30	14 days	2.0	Plants weakened
G4522 X Sun Dance	30	14 days	5.0	Plants died
Bioassay #3				
Corn control (Zapalote Chico)	30	11 days	4.0	Lodging (≧45°), leaf damage
Zapalote Chico X Tripsacorn	30	11 days	2.0	Minor leaf damage
Corn Control (W64A)	30	11 days	4.0	Lodging (≧45°)
W64A X Tripsacorn	30	11 days	2.3	Plant upright and growing
G4522 X Sun Dance	30	11 days	5.0	Lodging (≧45°), leaf damage
Tripsacorn	30	11 days	1.0	No evidence of

TABLE II-continued

RESULTS OF POT BIOASSAYS			
No. of Larvae	Duration	Root Damage*	Observations/Comments
			damage

*Hills and Peters scale (1971)

DEPOSIT OF SEEDS

Seeds derived from crosses between *Tripsacum dactyloides* and *Zea diploperennis* as described herein were deposited in accordance with the provisions of the Budapest Treaty with American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852 on Aug. 28, 1992. The accession number is ATCC75297.

The present invention is not limited in scope by the seeds deposited, since the deposited embodiments are intended as single illustrations of one aspect of the invention and any seeds, cell lines, plant parts, plants derived from tissue culture or seeds which are functionally equivalent are within the scope of this invention. While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that changes and modifications can be made without departing from the spirit and scope of the invention in addition to those shown and described herein. Such modifications are intended to fall within the scope of the appended claims.

I claim:

1. A method of producing a hybrid plant seed having a diploid chromosome number of between 18 and 20, comprising the steps of:

(a) crossing a *Tripsacum dacyloides* female parent with a *Zea diploperennis* male parent to produce a seed; then

(b) harvesting said seed produced in (a); wherein said seed has all of the identifying characteristics of ATCC 75297, and wherein a plant grown from said seed is a fertile hybrid.

2. Seed produced in accordance with the method of claim 1.

3. A hybrid plant grown from seed according to claim 2, said plant being fertile and having a diploid chromosome number of between 18 and 20.

4. Pollen produced by a plant according to claim 3.

5. A tissue culture produced from a plant according to claim 3, the cells of said tissue culture having a diploid chromosome number of between 18 and 20.

6. A method of producing a hybrid maize seed comprising the steps of:

(a) crossing a *Tripsacum dactyloides* female parent with a *Zer diploperennis* male parent to produce (*Tripsacum dactyloides X Zea diploperennis*) hybrid seed; then

(b) growing a (*Tripsacum dactyloides X Zer Diploperennis*) hybrid plant from said seed to maturity, then

(c) crossing said (*Tripsacum dactyloides X Zer diploperennis*) hybrid plant with Zea mays to produce seed;

(d) harvesting the seed produced in (c), wherein said seed produced in (a) all of has the identifying characteristics of ATCC 75297, and wherein said seed produced in (c) germinates into a plant having resistance to corn rootworm (*Diabrotica virgifera*).

7. Hybrid maize seed produced in accordance with the method of claim 6.

8. Hybrid maize plants grown from said seed of claim 7 which maize exhibits resistance to corn rootworm.

9. Hybrid maize plants grown from said seed of claim 7 which maize exhibits resistance to lodging.

10. Plants produced by in vitro propagation of said hybrid maize plants of claim 8, wherein said plants produced by in vitro propagation have resistance to corn rootworm (*Diabrotica virgifera*).

11. The method of claim 1, wherein said seed produced is ATCC 75297.

12. The method of claim 6, wherein said hybrid seed produced in step (a) is ATCC 75297.

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